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Probiotics for Preterm Infants: a strain specific systematic review and network meta-analysis.

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C.H.P. van den Akker, J.B. van Goudoever, and H. Szajewska proposed the idea of a network meta-analysis, were involved in the study design, selected appropriate studies, collected data, performed analyses, and wrote the manuscript.

N.D. Embleton, I. Hojsak, and R. Shamir were involved in the study design and interpretation of results, and critically reviewed and approved the latest version of the manuscript.

D. Reid developed software for this network meta-analysis and helped improving network models.

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Abstract

Objectives: Several randomised controlled trials (RCTs) on the use of probiotics to reduce morbidity and mortality in preterm infants have provided inconsistent results. Whilst meta-analyses that group all of the used strains together, suggest efficacy, it is not possible to determine the most effective strain which is more relevant to the clinician. We therefore used a network meta-analysis (NMA) approach in order to identify strains with greatest efficacy.

Methods: A PubMed search identified placebo-controlled or head-to-head RCTs investigating probiotics in preterm infants. From trials that recorded mortality, necrotising enterocolitis (NEC), late-onset sepsis (LOS), or time until full enteral feeding (TUFEF) as outcomes, data were extracted and Bayesian hierarchical random effects models were run to construct a NMA.

Results: Fifty-one RCTs involving 11,231 preterm infants were included. Most strains or combinations of strains were only studied in one or a few RCTs. Only 3 out of 25 studied probiotic treatment combinations showed significant reduction in mortality rates. Seven treatments reduced NEC incidence, 2 reduced LOS, and 3 reduced TUFEF. There was no clear overlap of strains which were effective on multiple outcome domains.

Conclusions: This NMA showed efficacy in reducing mortality and morbidity only in a minority of the studied strains or combinations. This may be due to an inadequate number, or size, of RCTs, or due to a true lack of effect for certain species. Further large and adequately powered RCTs using strains with the greatest apparent efficacy will be needed in order to more precisely define optimal treatment strategies.

Keywords: Premature neonates; necrotising enterocolitis; sepsis; enteral tolerance; microbiota

What is known:

- Several RCTs show that probiotics reduce neonatal morbidity and mortality, but data are inconsistent.
- Multiple different probiotic strains or combinations have been used in these RCTs.
- Most existing meta-analyses group different strains and fail to adequately account for strain specific effects.

What is new:

- Network meta-analysis shows that only a minority of probiotic strains have a statistically significant effect in reducing mortality and morbidity in preterm infants.
- The absence of significant effects may reflect a lack of adequately powered RCTs, or a genuine lack of efficacy for those species or strains.

Introduction

Necrotising enterocolitis (NEC) is a devastating disease in preterm infants strongly associated with gestational age, that has a variable incidence but typically averages 5-10% in infants weighing <1500 g (1). Mortality rates vary but range from 10 to 30%. Its pathogenesis is not completely understood as its occurrence may be the result of a variety of different aetiologies (2-4), and early detection is difficult (5-7). Accumulating evidence shows that in addition to the effect of human milk feeding (8-10), probiotics may be important (11) in preventing NEC and reducing mortality (12, 13).The role of the gut microbiota in the pathogenesis of NEC is, however, still unclear (14-16). Caution has therefore been advised until appropriately regulated safe products are available for use in this high-risk population (12, 16, 17). In its commentary published in 2010 on enteral nutrition in preterm infants the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition did not recommend routine probiotics administration in infants less than 1800 g (18).

Numerous meta-analyses have recently been published summarizing a large number of randomised clinical trials (RCTs) (19-32). In almost all of these meta-analyses the experimental group was constructed after pooling a wide variety of different probiotic strains as used in the original trials. This approach, however, does not give the clinician a meaningful answer as to which specific probiotic product has evidence-based efficacy. To partly overcome this problem, genus specific meta-analyses have been performed as well (25, 31). However, also within genera or species there might be significant differences in effectiveness depending on precise strain. Therefore, efficacy can only be evaluated at strain level and such meta-analyses have previously only been performed twice (21, 22).

Classical pair-wise meta-analyses address the comparative effectiveness among similar or competing interventions against a common comparator (usually placebo or standard care). In addition, these omit direct evidence from the few studies that provide head-to-head comparisons on probiotic strains. Network meta-analyses (NMA), however, can address multiple interventions simultaneously. This method allows a system to visualize and statistically combine evidence from direct comparisons with evidence from indirect comparisons across several competing interventions. By employing a NMA on data from RCTs of probiotics in preterm infants, our objective was to develop an updated and clinically meaningful understanding of the relative effectiveness of the different probiotic treatments.

Methods

Protocol registration and reporting guidelines

This systematic review was registered on PROSPERO (international prospective registry of systematic reviews) under number CRD42017064847. This manuscript is conducted and reported according to the methods and recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Extension Statement for Reporting of Systematic Reviews incorporating NMA (33).

Eligibility criteria

Inclusion criteria were studies that included only preterm infants, or studies that reported sub-groups between term and preterm infants so that only results from the latter could be included. Preterm birth was defined as gestational age of less than 37 weeks. Studies comparing probiotic treatment against placebo, usual care, or head-to-head with a different probiotic regime were considered eligible. If a study intervention consisted of the combination of a probiotic strain

together with another form of intervention, such as prebiotics or lactoferrin, the study was only included if a control group was included which received the same non-probiotic intervention (e.g. prebiotics or lactoferrin), but without the probiotic intervention. Studies could be included when infants were fed own mother's milk, donor milk or formula. Single or multiple strain studies were included. Studies were included if they reported well-described outcome reports of NEC (with Bell stages included (34-36)), blood-culture proven late-onset sepsis (LOS), postnatal age at reaching full enteral feeding (150 mL/kg per day), or in-hospital mortality. Other outcomes were not considered in this report. We included only RCTs (including cluster RCTs) which were fully published and in the English language. Complete blinding was not considered as mandatory. The results from non-randomised studies, conference papers, abstracts, or other non-published studies were not included.

Search strategy

A systematic literature search was conducted using the PubMed database from inception to September 19th, 2017. Search terms were: probiotic* AND (premature OR preterm OR neonat* OR infant*), with a limit on 'Clinical Trial'. In addition, reference lists of previously published meta-analyses were screened to identify additional eligible studies. The literature search was conducted independently by two reviewers (CHvdA and JBvG).Inconsistency whether to include a study was resolved by discussion.

Data extraction

Two round table meetings of the group (May 2016, Athens and May 2017, Prague) were held to achieve consensus on the approach, outcomes assessed, methods and to resolve all differences in interpretation of the methodological assessment and results of the eligible trials.

From the eligible studies information regarding inclusion criteria, study groups, key characteristics, and outcomes was extracted by the two reviewers independently using a standardized data collection form. Missing data were requested by contacting the authors. Probiotic strains were identified at strain levels (e.g. *Lactobacillusrhamnosus* GG ATCC 53103 or *Bifidobacteriumanimalis* subspecies *lactis* Bb-12), although often this was not available in the original reports. If no email reply was received from the original authors, strain numbers were obtained if possible through contacting manufacturers or internet searches on available product names.

For the remainder of this manuscript probiotic strains are truncated at their genus: *Bacillus, Bifidobacterium, Enterococcus, Lactobacillus, Saccharomyces,* and *Streptococcus* are denoted by *Ba., B., E., L.,Sa.,* and *S.*, respectively. In addition, subspecies (subsp) names are truncated as well: *B.animalis* subsp *lactis* is denoted as *B.lactis*; *B.bifidum* subsp *infantis* as *B.infantis*; *B.bifidum* subsp *longum* as *B.longum*, and *S. salivarius* subsp *thermophilus* as *S. thermophilus*. Over past decades multiple reclassifications in taxonomy have been proposed and designations in the historical publications may no longer be accurate. We therefore adhered to the latest nomenclature we were aware of. For example, *B.infantis* 35624 is designated as *B.longum* 35624 (37), *B.bifidum* Bb-12 as *B.lactis* Bb-12 (38), and *L.sporogenes* as *Ba.coagulans*(39). Since the *L.reuteri* strain DSM 17938 is a daughter strain of *L.reuteri* ATCC 55730 in which only resistance plasmids were removed but other characteristics are maintained (40), these strains are analysed together. Similarly, as *B.lactis* B94 shares many characteristics with *B.lactis* Bb-12 (41), these strains are also analysed together in our NMA. Except for in our study summary table 1, all control groups will be further denoted as placebo, whether true placebo was used, or whether usual care was given.

In case continuous data (i.e. data on time until full enteral feeding (TUFEF)) were originally presented as medians with corresponding interquartile percentiles or outer ranges, data were converted to estimated means with standard deviations, as previously described (42, 43). Obtained data were compared to previously published systematic reviews and discrepancies were rechecked in the original trials.

Assessment of study quality

To assess the methodological quality of the included RCTs the Cochrane Collaboration's tool for assessing risk of bias was used. The tool includes the following criteria: adequacy of sequence generation, allocation concealment, blinding of participants, personnel and outcome assessors, incomplete outcome data, selective reporting, and other biases (43). Noteworthy, studies were not excluded based on these results. Items were scored as low, high, or unknown risk of bias. Because of possible probiotic cross colonization from intervention to control groups, the item on other biases was scored as unknown, except in case of cluster RCTs. Publication bias was assessed by constructing funnel plots for each outcome.

Statistical analyses

Studies were compared between every studied probiotic strain or combination of studied strains via a comprehensive NMA based on the Bayesian theorem (44, 45). This approach can be considered to be an extension of the traditional pair-wise meta-analysis, as it incorporates both direct and indirect information through a common comparator (most often placebo or routine care) to obtain estimates of the relative interventional effects on multiple intervention comparisons.

Network graphs were constructed for each outcome variable. They consisted of nodes (points representing the competing interventions) and edges (adjoining lines between the nodes that

show which interventions have been compared among the included studies). The size of the nodes represent the number of infants that received the intervention. The thickness of the edges represent the number of comparative studies between the respective nodes. Reviewing the network geometry graphically summarizes how the evidence base is built up and whether various strains were directly compared or only through indirect network evidence.

We made use of the Aggregate Data Drug Information System 2 (ADDIS2) software (46, 47), which is an open-source online application based on R statistical software (48). The NMA was conducted using a Bayesian framework in combination with a Markov chain Monte-Carlo simulation. Given that most treatments included a limited number of RCTs, we assumed between-study heterogeneity, although this could not formally be tested. Therefore, a conservative random effect approach was employed in our NMA models.The NMA was run with an outcome scale of 5 to set default prior distributions accordingly. Run-lengths were based on at least 80000 inference iterations for each 4 chains with a burn-in period of the first 10000 iterations combined with a thinning factor of 10. Convergence was assessed using the Brooks-Gelman-Rubin diagnostic to reassure that all potential scale reduction factors (PSRFs) remained below 1.05. In the rare occasion that the PSRF approached 1.05 or was higher than 1.05, run-length was increased accordingly to improve model convergence.

Relative treatment effect plots were constructed for each studied probiotic strain (or combination of multiple strains) versus placebo. Dichotomous outcomes are expressed as risk ratios (RRs) with their 95% credible interval (95% CrI). Continuous outcome measures are expressed as mean differences with their 95% CrI.

Besides the relative effect plots in the NMA, we constructed classic pair-wise forest plots for those strains or combinations which were compared to placebo if they showed either significant

efficacy in the NMA or were evaluated in at least two RCTs and at least 250 infants were included in the treatment arm. These forest plots were constructed in RevMan (49) and visualise how the NMA evidence base is built up.

Consistency of the NMA model between direct and indirect network evidence was tested with the node splitting method (50). A known drawback from this approach, however, is that it cannot properly handle multi-arm studies (44). To give insight in possible publication biases, we constructed funnel plots for all studies that compared placebo versus any probiotic strain or combination for each outcome separately.

Results

Our PubMed search yielded 515 citations; a flow diagram on the screening and eligibility process is presented in figure 1. In total 56 articles (for 49 different RCTs) were identified which fulfilled our inclusion criteria and reported usable extractable outcome data on at least one of our predefined outcome domains (51-106). Five of these articles only showed overlapping data and were further discarded (102-106). Two studies were either a sub-study (65) from a multicentre study (51), or an extension (75) of an already published trial (74); from the sub-studies only data not elsewhere reported was entered in our database. One small RCT only described that NEC, sepsis, and mortality incidence were not different between groups (107), but no response was received from the authors after requesting original data. Eight studies were identified with appropriate study design and inclusion criteria, although no data on our outcome domains were described (108-113) or only long-term follow-up data was presented (114, 115). In 4 studies, the use of probiotics was not the only difference between intervention and control groups, i.e. prebiotics were used only in the intervention group (116, 117), or only the control group received

nystatin instead of placebo (118, 119). One study included both preterm and term neonates and results were not split between subgroups (120). The studies from which no suitable data on our outcome domains could be extracted, were therefore excluded from further analysis in this report (107-120). From the 51 included articles (51-101), data from 11,231 preterm infants could potentially be extracted. Table 1 shows study details and main characteristics of the included patients. Based on broadly overlapping basic inclusion criteria and patient characteristics across the various trials we assumed transitivity (33, 44). Identified strain numbers are summarized in appendix table S1 (Supplemental Digital Content, http://links.lww.com/MPG/B273). Unfortunately, we were not able to retrieve them for all microorganisms. Results for which no strain number is available must therefore be interpreted with caution as different products might have been used. Appendix figures S1A&B (Supplemental Digital Content, http://links.lww.com/MPG/B273) show the risk of bias assessment.

Mortality

Figure 2 shows the network graph comparing the probiotic strains or combinations as used in the original trials for the reduction of in-hospital mortality. The network geometry shows the evidence base comparing 25 different treatments (n=4788 in total in 39 arms; mortality incidence 5.1%) versus the common comparator placebo (n=4512 in 36 trials; mortality incidence 7.0%). Original mortality incidence data from all included studies is shown in appendix table S2 (Supplemental Digital Content, http://links.lww.com/MPG/B273). There were only 4 direct head-to-head comparisons. Three interventions had to be excluded from the quantitative analysis because there were zero events in the placebo or intervention groups which is not compatible with NMA analyses. In one study using *Sa.boulardii* CNCM I-3799 (100), there were no mortality cases in both the intervention and control groups; Three studies (2 treatments:

B.bifidum OLB6378 (95); and *B. breve* M-16V (67, 81)) were excluded because there were zero events in the intervention groups, although mortality rates in the placebo groups from these studies amounted 2/153 and 4/176, respectively (events/N).

Figure 3 shows the relative effects plot for efficacy in reducing mortality of the various tested probiotic strains or tested combinations versus placebo treatment. It shows that the RRs for mortality are significantly reduced for 3 interventions: for the combination of *B.bifidum* NCDO 1453 and *L.acidophilus* NCDO 1748 (based on 2 studies with 494 infants (72, 87)); the combination of *B.bifidum*, *B.infantis*, *B.longum*, and *L.acidophilus* (based on 1 study with 186 infants (88)); and the combination of *B.infantis*, *L.acidophilus*, *L.casei*, *L.plantarum*, *L.rhamnosus*, and *S.thermophilus* altogether (based on 1 study with 150 infants (63)); see also table 2. Separate pair-wise forest plots are shown in appendix figure S2 (Supplemental Digital Content, http://links.lww.com/MPG/B273) for probiotic strains or combinations which were significant or were tested in at least 250 infants versus placebo. Node-splitting detection of inconsistency was not possible in this model due to only head-to-head trials from multiple arm studies, or head-to-head comparisons with 0 events in one or more study arms (50). A funnel plot shows no clear evidence of a publication bias (appendix figure S3, Supplemental Digital Content, http://links.lww.com/MPG/B273).

Necrotising enterocolitis

Figure 4 shows the network graph comparing the probiotic strains or combinations as they were used in the original trials for the prevention of NEC grades 2 or 3. The network geometry shows the evidence base comparing 25 different treatments (n=5550 in total in 50 arms; NEC incidence 3.2%) versus the common comparator placebo (n=5101 in 43 trials; NEC incidence 6.1%). Original NEC incidence data from all included studies is shown in appendix table S3

(Supplemental Digital Content, http://links.lww.com/MPG/B273). There were only 6 direct head-to-head comparisons. Three studies (using *Ba.clausii* 4 strains (94); *B.bifidum* OLB6378 (95); and *Sa.boulardii* CNCM I-3799 (100)) had to be excluded from the NMA because there were zero events in both the placebo and intervention groups. Seven studies (4 treatments: *B.breve* M-16V (64, 67, 81, 98); *B.breve* and *L.casei*(55); *B.infantis* PTA-5843, E. *faecium* PTA-5844, and *L.gasseri* PTA-5845 (70); and *L.acidophilus* Lb (53)) were excluded because there were zero events in the intervention groups, although NEC rates in the placebo groups from these studies amounted 1/127, 4/112, 5/40, and 5/16, respectively (events/N).

Figure 5 shows the relative effects plot for efficacy in reducing NEC grade 2 or 3 of the various tested probiotic strains or tested combinations versus placebo treatment. It shows that the RRs for NEC are significantly reduced for 7 treatments: *B.lactis* Bb-12 or B94 (based on 5 trials with 828 infants (61, 66, 76, 78, 93)); *L.reuteri* ATCC 55730 or DSM 17938 (based on 4 studies with 1459 infants (79, 83, 84, 91)); *L.rhamnosus* GG (based on 6 studies with 1507 infants (56, 59, 73, 75, 84, 96)); the combination of *B.bifidum*, *B.infantis*, *B. longum*, and *L.acidophilus* (based on 2 studies with 247 infants (88, 96)); the combination of *B.infantis* ATCC 15697 and *L.acidophilus* ATCC 4356 (based on one study with 367 infants (71)); the combination of *B.infantis* Bb-02, *B.lactis* Bb-12, and *S.thermophilus* TH-4 (based on 2 studies with 1244 infants (54, 69)); and the combination of *B.longum* 35624 and *L.rhamnosus* GG (based on 2 studies with 285 infants (51, 97)); see also table 2. Separate pair-wise forest plots are shown in appendix figure S4 (Supplemental Digital Content, http://links.lww.com/MPG/B273) for probiotic strains or combinations which were significant or were tested in at least 250 infants versus placebo.

Node-splitting models did not show substantial differences between direct and indirect evidence, so that the consistency model holds (appendix figure S5, Supplemental Digital Content,

http://links.lww.com/MPG/B273) (50). The funnel plot shows no clear evidence of a publication bias (appendix figure S6, Supplemental Digital Content, http://links.lww.com/MPG/B273).

Late-Onset Sepsis

Figure 6 shows the network graph comparing the probiotic strains or combinations as they were used in the original trials for the prevention of culture proven LOS. The network geometry shows the evidence base comparing 25 different treatments (n=5576 in total in 52 arms; sepsis incidence 15.4%) versus the common comparator placebo (n=5049 in 45 trials; sepsis incidence 24.9%). Original LOS incidence data from all included studies is shown in appendix table S4 (Supplemental Digital Content, http://links.lww.com/MPG/B273). There were only 6 direct head-to-head comparisons. One intervention using *Sa.boulardii* CNCM I-3799 had to be excluded because there were zero events in both the placebo and intervention groups (100). Figure 7 shows the relative effects plot for efficacy in reducing LOS of the various tested probiotic strains or tested combinations versus placebo. It shows that the RRs for LOS are significantly reduced for 2 probiotic treatments: for the combination of *B.bifidum*, *B.infantis*, *B. longum*, and *L.acidophilus* (based on 2 studies with 247 infants (88, 96)); and for the combination of *B. longum* R00175, *L. helveticus* R0052, *L. rhamnosus* R0011, and *Sa. boulardii* CNCM I-1079(based on 3 studies with 241 infants (52, 62, 92)); see also table 2. Separate pair-wise forest plots are shown in appendix figure S7 (Supplemental Digital Content, http://links.lww.com/MPG/B273) for probiotic strains or combinations which were significant or tested in at least 250 infants versus placebo. Node-splitting models did not show substantial differences between direct and indirect evidence, so that the consistency model holds (appendix figure S8, Supplemental Digital Content, http://links.lww.com/MPG/B273) (50). The funnel plot

shows no clear evidence of a publication bias (appendix figure S9, Supplemental Digital Content, http://links.lww.com/MPG/B273).

Time until full enteral feeding

Figure 8 shows the network graph comparing the probiotic strains or combinations as they were used in the original trials to reduce TUFEF. The network geometry shows the evidence base comparing 13 different treatments (n= 3122 in 24 arms) versus the common comparator placebo (n=2988 in 21 trials). There were only 2 direct head-to-head comparisons. Original data from all included studies is shown in appendix table A5 (Supplemental Digital Content, http://links.lww.com/MPG/B273). Figure 9 shows a relative effects plot for efficacy in reducing TUFEF of the various tested probiotic strains or tested combinations versus placebo treatment. It shows that the mean difference for TUFEF is significantly reduced for 3 interventions: *L.reuteri* ATCC 55730 or DSM 17938 (based on 3 studies with 626 infants (79, 84, 91)); and for the combination of *B.bifidum*, *B.infantis*, *B. longum*, and *L.acidophilus* (based on 2 studies with 247 infants (88, 96)); and for the combination of *B.longum* BB536 and *L.rhamnosus* GG (based on 1 study with 94 infants (85)); see also table 2. Separate pair-wise forest plots are shown in appendix figure S10 (Supplemental Digital Content, http://links.lww.com/MPG/B273) for probiotic strains or combinations which were significant or tested in at least 250 infants versus placebo. Node-splitting models did not show substantial differences between direct and indirect evidence, so that the consistency model holds (appendix figure S11, Supplemental Digital Content, http://links.lww.com/MPG/B273) (50). From the funnel plot, a publication bias could not be excluded, as no clear triangular shape could be identified from included studies (appendix figure S12, Supplemental Digital Content, http://links.lww.com/MPG/B273).

Discussion:

By using the approach of a NMA, we were able to determine, based on the current literature, which tested probiotic strains were most effective, and which were not, in reducing mortality and morbidity in preterm infants. Only 3 out of 25 studied probiotic treatments showed significant reduction in mortality rates. Seven treatments reduced NEC incidence, 2 reduced LOS, and 3 reduced TUFEF. There was no clear overlap of certain strains which were significantly effective on multiple outcome domains. Most strains or combination of strains only showed trends towards efficacy, whereas other strains did not demonstrate efficacy (such as *Sa.boulardii* CNCM I-745 and*B.breve* BBG-001). A lack of effect may either be due to understudied species or a true lack of effect of certain strains.

Although the total number of 51 RCTs included with over 11,000 infants is considerable (51-101), many of the different probiotic treatments were only evaluated in one or two trials. Only five strains were studied in at least 4 RCTs (see also tables S2-5): *B.breve* M-16V, *B.lactis* Bb-12 or B94, *L.reuteri* ATCC 55730 or DSM 17938, *L.rhamnosus* GG ATCC 53103, and *Sa.boulardii* CNCM I-745. In addition, the evidence base was frequently dependant on small, lower quality, or outdated studies as can be seen in the pair-wise forest plots. We chose to include moderately preterm infants in our analyses noting that the number of studies which focussed on the smallest infants was very limited. In only 4 of the 51 included studies (compromising 3 different RCTs), the mean birth weight was below 1000 g (table 1) (51, 65, 76, 97). In 10 studies, the mean birth weight was at least 1500 g. There were many studies with unclear risks of bias for the various domains, and 8 studies with high risks in at least 1 domain (64, 67, 70, 82, 84, 87, 98, 101). However, most of the studies with a high risk of bias assessment did not contribute to the significant strains. An unclear risk indicates that the risk item was not

clearly described, but does not necessarily indicate a potentially flawed study. We decided not to exclude studies purely on the basis of the quality assessment criteria, but allow readers to make their own interpretation of the evidence base. A visual inspection of the evidence bases by means of the funnel plots did not show a clear publication bias for most outcomes, although for TUFEF no triangular shape could be identified.

In most of the original studies the primary outcome was not on one of our outcome domains (table 1), but on for example stool colonization, growth rates, or not reported even more frequently. If one performs a power calculation (α 5%; 1-β 80%) on reducing mortality rates while taking the observed rates in our manuscript (7.0 vs 5.1%), one would need almost 2500 infants for each studied strain. For NEC, to demonstrate a reduction from 6.1 to 3.2%, one would need more than 800 infants per group. These high inclusion rates have not been reached for these outcomes. One must therefore realise that the results here presented are based on exploratory data analysis and thus only have hypothesis generating power.

It must be noted that some interesting results were produced. For example, both *L.rhamnosus* GG and *B.lactis* Bb-12 / B94 appeared to be effective in reducing NEC (figure 5 and figure S4). In addition, *B.longum* BB536 showed a clear trend towards a similar effect. However, both the combination of *L.rhamnosus* GG with *B.longum* BB536 and the combination of *B.lactis* Bb-12 with *B.longum* BB536 showed no measurable effect. This may reflect an antagonistic effect of *B.longum* BB536 together with the other two strains, or the relatively poor evidence base on which this NMA is built. A similar pattern was seen with these strains in the reduction of LOS, although much less pronounced. Somewhat un-expectedly, only *L.rhamnosus* GG simultaneously administered with *B.longum* BB536 was able to reduce TUFEF significantly

(based on 1 study with 94 infants studied (85)), whereas *L.rhamnosus* GG alone was not (figure 9 and figure S10).

The classic strain versus placebo forest plots show that the effect size was more or less similar to evidence from relative effect plots in the NMA. The small differences can be explained due to the Bayesian statistical approach versus the classic frequentist random-effects models, but it is known that Bayesian techniques better account for trial heterogeneity. In addition, the NMA gained extra power from indirect network evidence, from studies in which there was no suitable placebo group but only provided head-to-head comparisons (96, 101), and from studies in which data from multiple treatment arms could be included (66, 84). Unfortunately, most network evidence was based on indirect comparisons as the vast majority only compared treatment versus placebo or routine care. There were only a few head-to-head trials available that could be tested for inconsistency by means of the node splitting method. Nevertheless, inconsistency between direct and indirect network evidence was not apparent in tested cases.

As is the case for almost any meta-analysis, there are small differences in study design in terms of inclusion criteria (e.g. birth weight, gestational age, degree of growth restriction) or drug administration regimens (initiation, duration, and dosing). Although most studies did not exclude infants depending on their dietary exposures (formula, own mother's milk, or donor milk), some studies only included infants who either received only breast milk, or only formula. The magnitude of how this affected results is unknown. Nevertheless, transitivity was assumed as inclusion criteria were all broadly overlapping. A further bias could be that we included only RCTs published in the English language due to language barriers for reviewing. On the other hand, most RCTs from other settings such as for example China used different combinations of strains that were not tested in the RCTs included here (29). A major strength of our analysis, is

that we paid meticulous attention to retrieve the correct probiotic strain in the included RCTs. Regrettably, strain numbers were frequently not mentioned in the original manuscripts and could sometimes not be retrieved despite contacting original authors or companies. The results from species without further designation should therefore be interpreted with caution. It is no longer acceptable in current studies to omit a clear description of the used probiotic drug at subspecies level with strain number according to the latest taxonomic nomenclature (17, 121). In addition, many commercial products turned out to contain different bacterial strains than were included on the ingredient list (17, 122). Future studies should therefore validate their studied probiotic strains and exclude contamination by other strains. Apart from efficacy, a high degree of quality control and assurance is mandatory as probiotic related sepsis has been regularly reported in preterm infants who can be considered immuno-compromised patients (17, 31). An additional safety issue could be that some probiotics strains carry antibiotic resistance genes themselves, and could thus have the potential to pass the antibiotic resistance genes to pathogenic bacteria through horizontal gene transfer (123). These elements need to be taken into account when balancing supposed or true beneficial and harmful effects.

To conclude, our efforts in this study were to present an overview of all published evidence on the use of probiotics in preterm infants at a strain level, and to identify the most promising strains. Most strains were unfortunately only studied once or a few times. In addition, the number of reports in the most preterm neonates was very limited. Furthermore, it was not possible to determine optimal probiotic dosages, time of initiation, and duration of treatment course. Nevertheless, we believe that our approach of a strain-specific NMA gives a much more meaningful answer than previously performed meta-analyses in which all probiotic strains were analysed as one group, or were grouped at a genus or species level, as even these latter analyses

do not address strain specific characteristics. The NMA allowed us to identify potential strains that can reduce NEC and mortality incidence in this vulnerable population. Still, our major and rather disappointing conclusion, is that more than 10 years from the first RCTs showing that probiotics may reduce a disease as serious as NEC, we remain unable to clearly identify the optimal strain, dose or combination, and that clinicians are left using inadequately tested, potentially un-safe and possibly ineffective treatments.

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Figure legends:

Figure 1: Flow diagram of search and inclusion strategy.

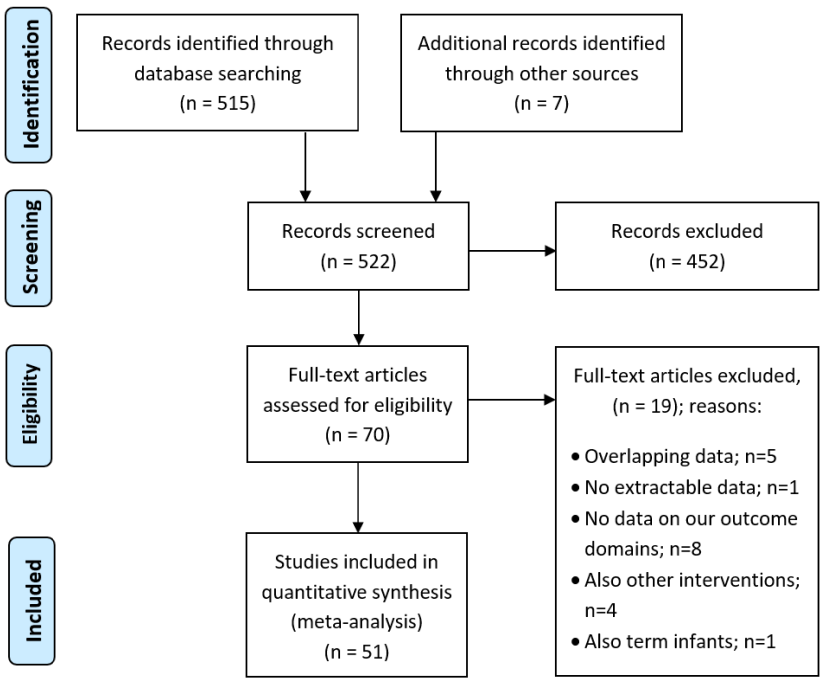

Figure 2: Network graph of all tested probiotic strains or combinations thereof in the reduction of in-hospital mortality.* *B. longum* R00175, *L. helveticus* R0052, *L. rhamnosus* R0011, and *Sa. boulardii* CNCM I-1079. ***B. infantis, L. acidophilus, L. casei, L. plantarum, L rhamnosus,* and *S. thermophilus*.

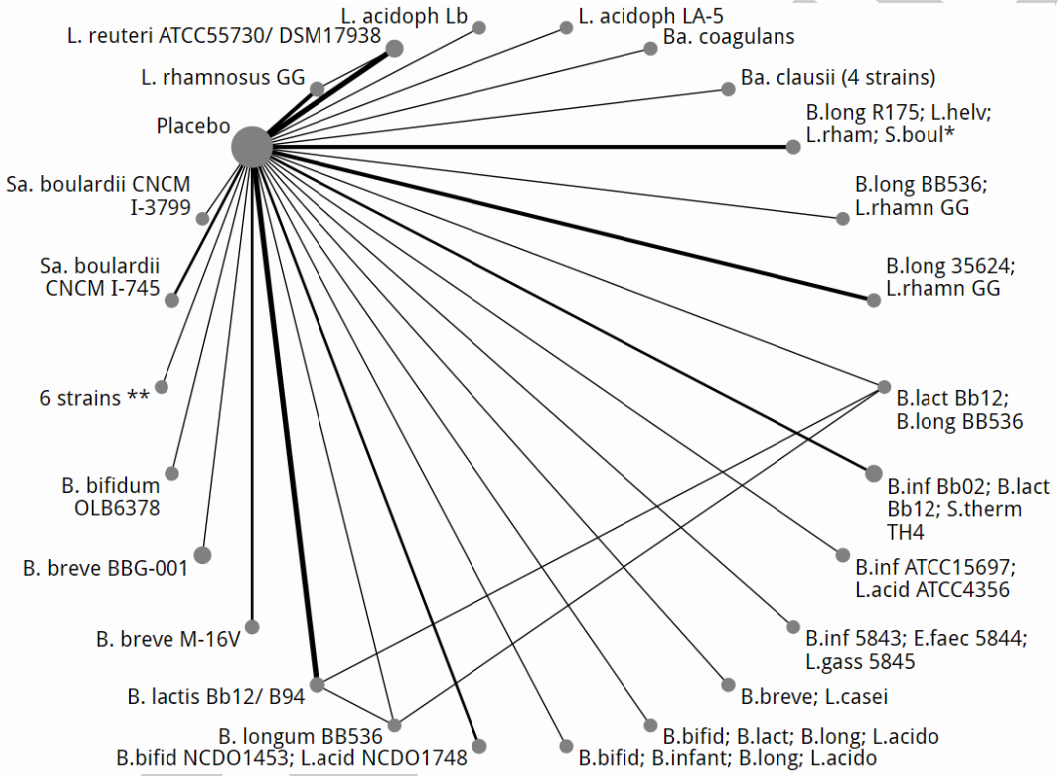

Figure 3: Relative effects plot for reduction of mortality. * *B. longum* R00175, *L. helveticus* R0052, *L. rhamnosus* R0011, and *Sa. boulardii* CNCM I-1079. ***B. infantis, L. acidophilus, L. casei, L. plantarum, L rhamnosus,* and *S. thermophilus*.

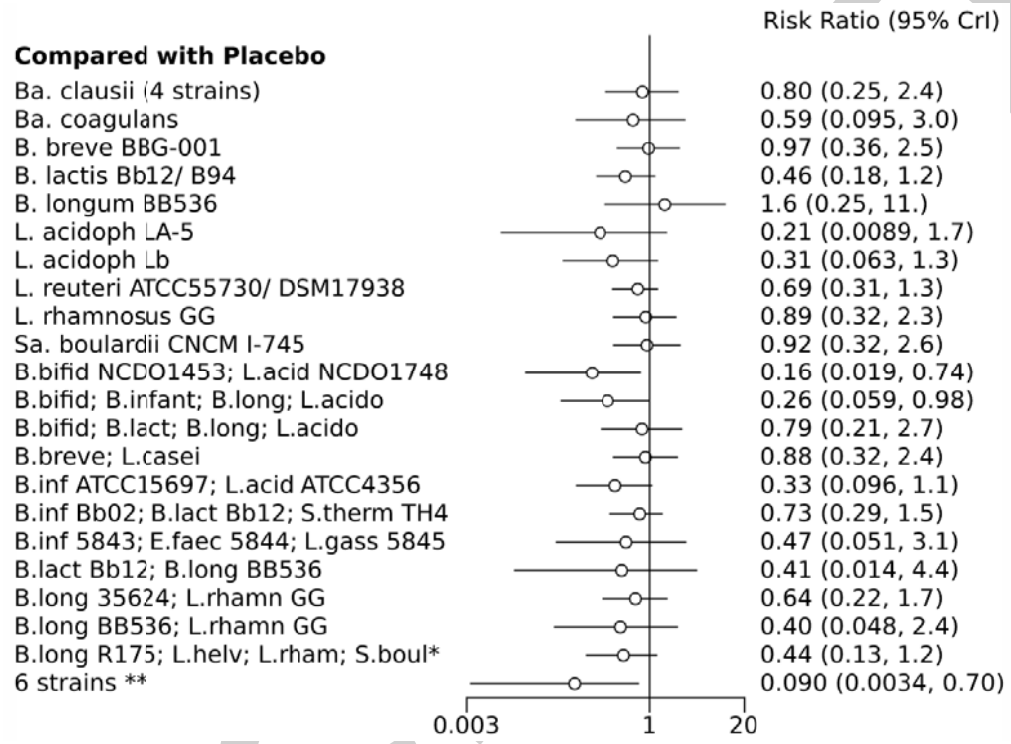

Figure 4: Network graph of all tested probiotic strains or combinations thereof in the reduction of NEC grades 2 or 3. * *B. longum* R00175, *L. helveticus* R0052, *L. rhamnosus* R0011, and *Sa. boulardii* CNCM I-1079. ***B. infantis, L. acidophilus, L. casei, L.*

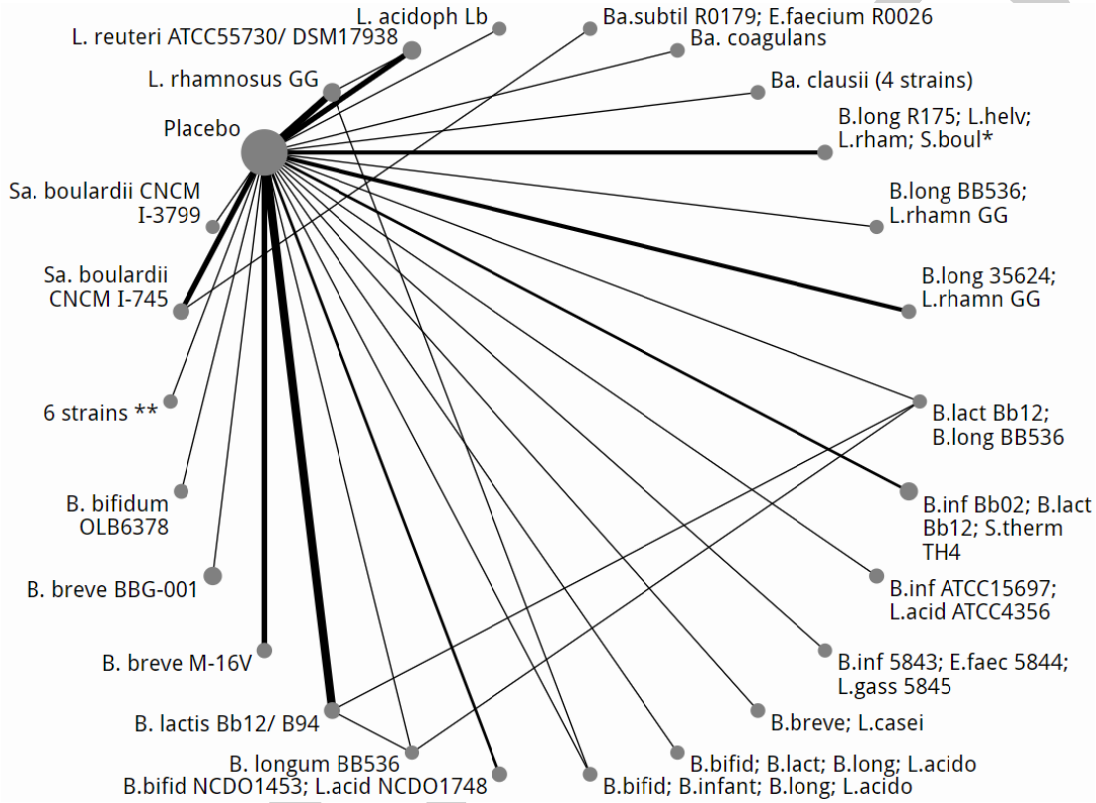

Figure 5: Relative effects plot for reduction of NEC grades 2 or 3. * *B. longum* R00175, *L. helveticus* R0052, *L. rhamnosus* R0011, and *Sa. boulardii* CNCM I-1079. **B. infantis, L. acidophilus, L. casei, L. plantarum, L rhamnosus,* and *S. thermophilus*. *plantarum, L rhamnosus,* and *S. thermophilus*.

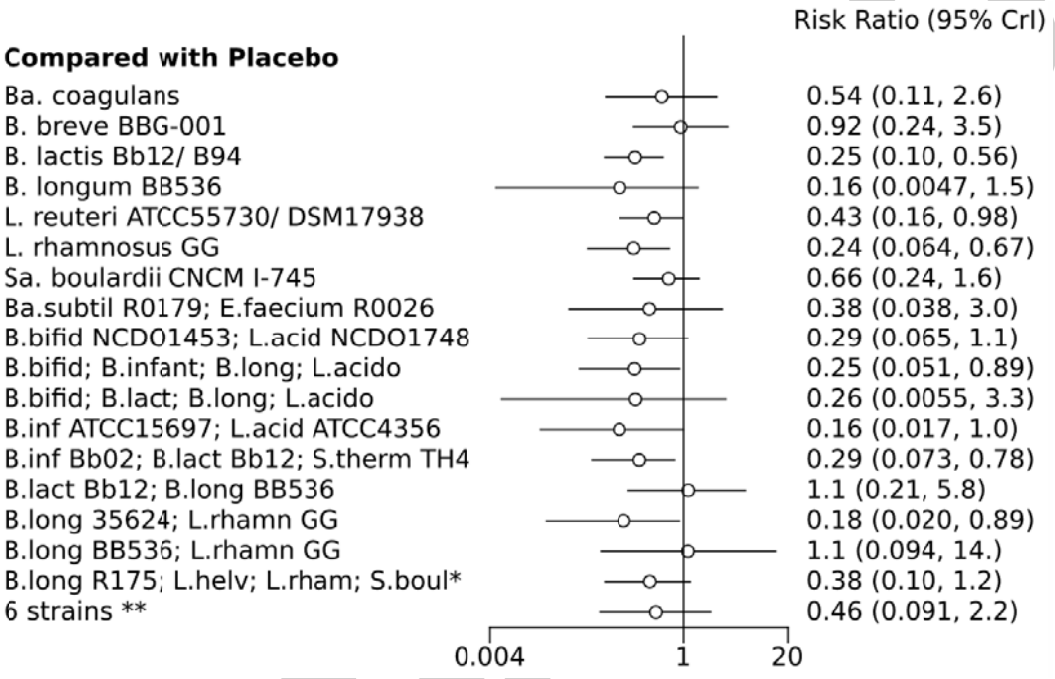

Figure 6: Network graph of all tested probiotic strains or combinations thereof in the reduction of LOS.* *B. longum* R00175, *L. helveticus* R0052, *L. rhamnosus* R0011, and *Sa. boulardii* CNCM I-1079. ***B. infantis, L. acidophilus, L. casei, L. plantarum, L rhamnosus,* and *S. thermophilus*.

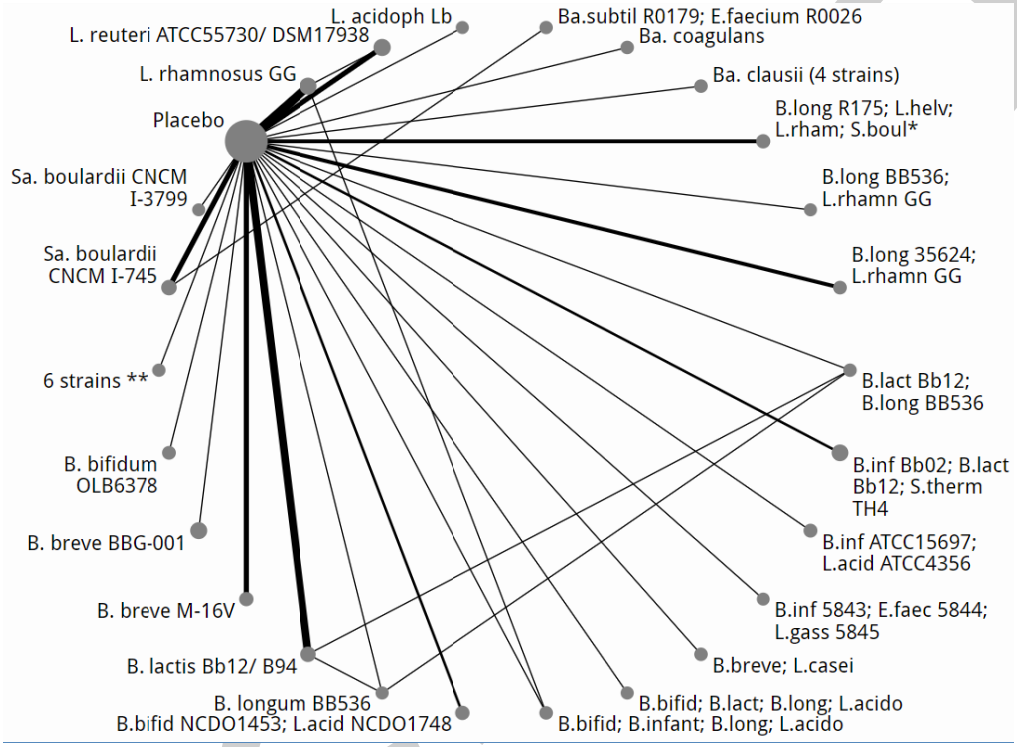

Figure 7: Relative effects plot for reduction of LOS. * *B. longum* R00175, *L. helveticus* R0052, *L. rhamnosus* R0011, and *Sa. boulardii* CNCM I-1079. ***B. infantis, L. acidophilus, L. casei, L. plantarum, L rhamnosus,* and *S. thermophilus*.

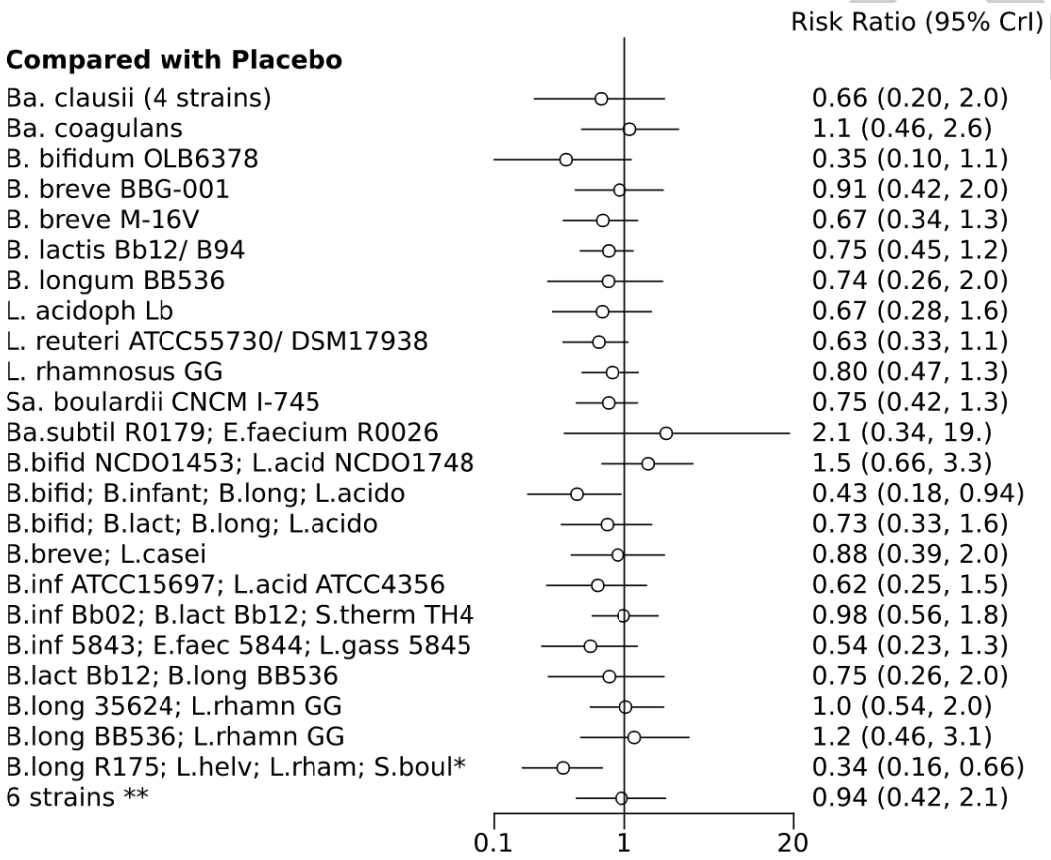

Figure 8: Network graph of all tested probiotic strains or combinations thereof in the reduction of time to reach full enteral feeding.* *B. longum* R00175, *L. helveticus* R0052, *L. rhamnosus* R0011, and *Sa. boulardii* CNCM I-1079.

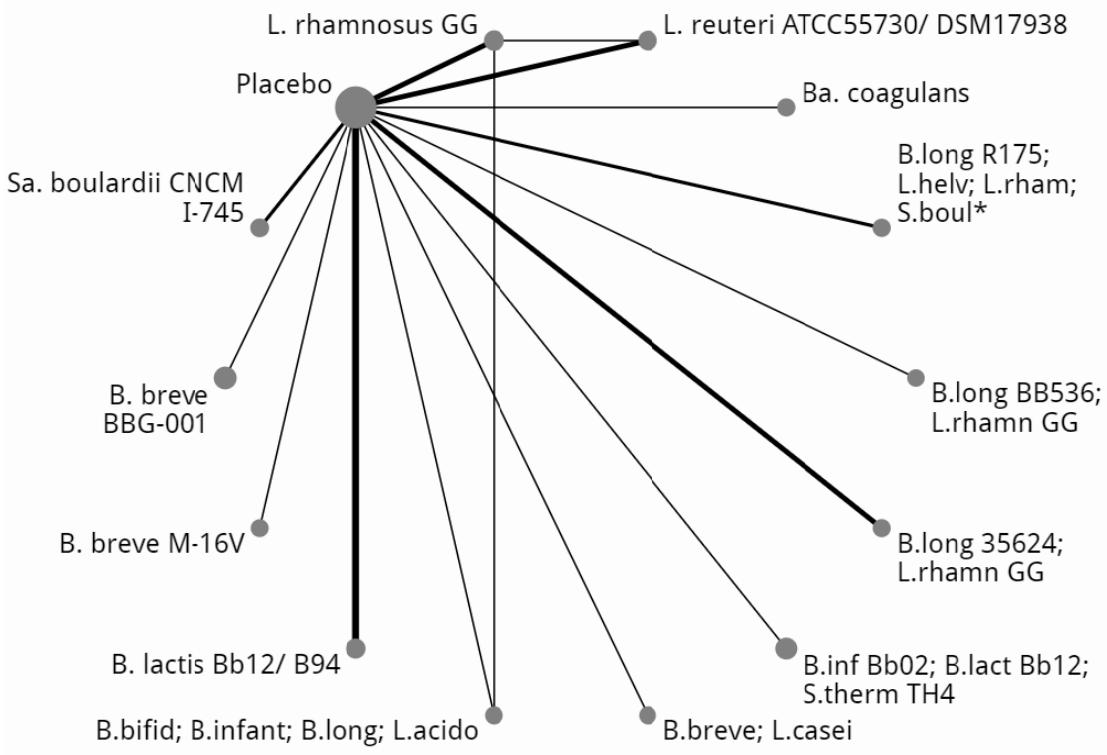

Figure 9: Relative effects plot for reduction of time to reach full enteral feeding (d).* *B. longum* R00175, *L. helveticus* R0052, *L. rhamnosus* R0011, and *Sa. boulardii* CNCM I-1079.

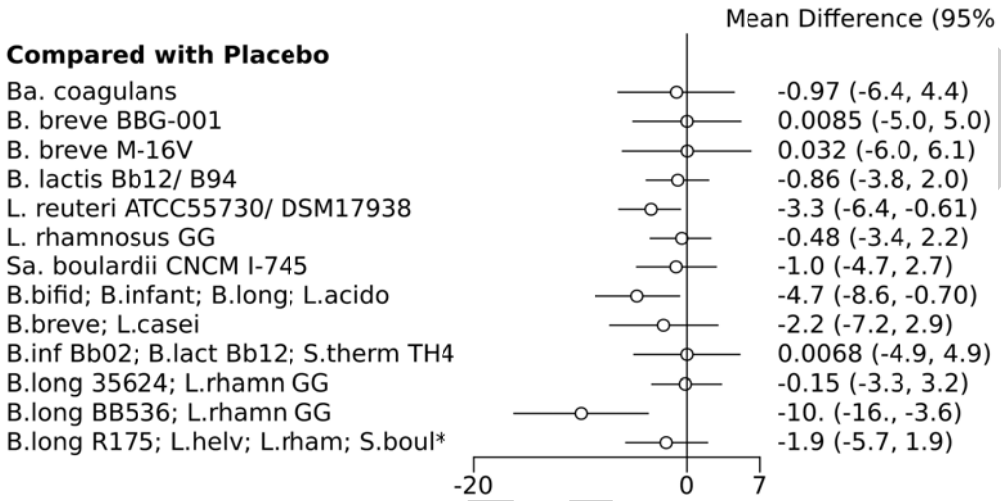

Table 1: Overview of included studies with their design, who prepared the control and intervention drugs, main inclusion criteria, feeding modality, duration of intervention, used probiotic strains including dose and manufacturer if provided, included number of patients (N), average gestational age (GA) and birth weight (both reported as mean and SD unless specified otherwise §,#,$), outcome on which power calculation was performed, and reported outcomes of interest.

Ref	Study (first author, year, country, # sites)	Design	Study preparation	Main inclusion criteria	Feeding modality	Duration of intervention	Groups (incl daily dose and manufacturer)	N	Average GA (wks)	Average weight (g)	Power calculation	Reported outcomes
51	Al-Hosni, 2012, USA, 3	DB	Pharmacy or nutritional nurse	Preterm, 501-1000 g, AGA	NR	First enteral feeding until discharge or 34 wks PMA	- Control (unsupplemented milk) - *B. longum* 35624 (5×10^8 CFU; Align) and *L. rhamnosus* GG ATCC 53103 (5×10^8 CFU; Culturelle)	- 51 - 50	- 25.7 ± 1.4 - 25.7 ± 1.4	- 779 ± 126 - 778 ± 138	Discharge weight <p10	NEC 1, NEC 2, NEC 3, NEC 2 or 3, any NEC, sepsis, and mortality
52	Arora, 2017, India, 1	DB *	NR	≤34 wks	OMM	Start when enteral feeds were tolerated, durations 2 weeks	- Control (unsupplemented milk) - *B. longum* R00175, *L. helveticus* R0052, *L. rhamnosus* R0011, and *Sa. boulardii*	- 75 - 75	-32.9 ± 1.2 (all infants together) *	- 1700 ± 287 (all infants together) *	NR	NEC 1, NEC 2, NEC 3, NEC 2 or 3, any NEC, sepsis, TUFEF*, and mortality

							CNCM I-1079 (2.5×10^9 CFU); Darolac, Aristo *					
53	Awad, 2010, Egypt, 1	DB	NR	Preterm neonates at NICU, low CRP, neg BC	NR	Until discharge	- Control (water in opaque tube) - *L. acidophilus* Lb (1.2×10^{10} CFU); Lacteol fort, Axcan Pharma	- 16 - 36	- 33.8 ±1.8 - 33.0 ± 2.8	- 2100 ± 750 - 1830 ± 520	NR	NEC 2 or 3, sepsis, and mortality
54	Bin-Nun, 2005, Israel, 1	DB	Study staff	Preterm, <1500 g	OMM or PF	Start feedings until 36 wks PCA	- Control (unsupplemented milk) - *B. lactis* Bb-12 [1] (3.5×10^8 CFU), *B. infantis* Bb-02 (3.5×10^8 CFU), and *S. thermophilus* TH-4 (3.5×10^8 CFU); ABC Dophilus	- 73 - 72	-29.3 ± 4.3 - 29.8 ± 2.6	- 1111 ± 278 - 1152 ± 262	NEC (w/o grade)	NEC 1, NEC 2, NEC 3, NEC 2 or 3, any NEC, sepsis, TUFEF, and mortality
55	Braga, 2011,	DB	Nutrition assistants	750 – 1499 g	OMM, DM, or	DOL 2 until DOL	- Control - *B. breve*	- 112	- 29.2 ± 2.6	- 1151 ± 225	NEC ≥ 2	NEC 2 or 3, sepsis, TUFEF,

	Brazil, 1				PF	30	(5x10^{7-9} CFU)and*L. casei* (2×10^{7-9} CFU); Yakult LB	- 119	- 29.5 ± 2.5	- 1195 ± 206		and mortality
56	Chrzanowska-Liszewska, 2012, Poland, 1	DB, PC	Pharma-ceutical company	>1000 g and <32 wks	PF	Start not mentioned. Duration 42 study days	- Placebo - *L. rhamnosus* GG ATCC 53103 (6×10^{9} CPU); Vitis Pharma	- 26 - 21	- 29.5 - 29.6	- 1283 - 1227	LGG positive stool culture	Sepsis
57	Costalos, 2003, Greece, 1	DB, PC	NR	28-32 wks, no antibiotics	PF	Started when feedings. Median duration 30 days	- Placebo - *Sa. boulardii* CNCM I-745 (2×10^{9} CFU)	- 36 - 51	- 31.8 - 31.1§	- 1644 - 1651§	'intestinal flora'	NEC (grade not specified)**, sepsis, andTUFEF
58	Costeloe, 2016, England, 24	DB, PC	Milk kitchen	23-30+6 wks	OMM, DM, or PF	Start within 48h, until 36 wks PMA	- Placebo - *B. breve* BBG-001 (8.5×10^{10} CFU); Yakult Honsha	- 660 - 650	- 28.0 (26.1-29.6) - 28.0 (26.1-29.4) #	- 1043 ± 317 - 1039 ± 312	NEC ≥2, sepsis, or death	NEC 2, NEC 3, NEC 2 or 3, sepsis, TUFEF, and mortality
59	Dani, 2002, Italy, 12	DB, PC	NR	<33 wks or <1500 g	OMM, DM, or PF	First feed, until discharge	- Placebo - *L. rhamnosus* GG ATCC 53103 (6×10^{9} CFU); Dicoflor	- 290 - 295	- 30.7 ± 2.3 - 30.8 ± 2.4	- 1345 ± 384 - 1325 ±361	NR	NEC 2 or 3, and sepsis
60	Demirel, 2013,	DB	Milk kitchen	≤32 wks and	OMM, or PF	First feed, until	- Control (unsuppleme	- 136	- 29.2 ± 2.5	- 1131 ± 284	NEC ≥2 or death	NEC 2, NEC 3, NEC 2 or 3,

	Turkey, 1			≤1500 g		discharge	nted milk) - *Sa. boulardii* CNCM I-745 (5×10^9 CFU); Reflor	- 135	- 29.4 ± 2.3	- 1164 ± 261		sepsis, TUFEF, and mortality
61	Dilli, 2015, Turkey, 5	DB, PC	Pharma-ceutical company	<32 wks and <1500 g	OMM or PF	Start at DOL 7, until discharge or max 8 wks	- Placebo - *B. lactis*B94 * (5×10^9 CFU); Maflor - Inuline - Inuline + *B. lactis*B94 * (5×10^9 CFU); Maflor	- 100 - 100 - 100 - 100	- 28.2 ± 2.2 - 28.8 ± 1.9 - 29.0 ± 1.7 - 28.9 ± 1.9	- 1147 ± 271 - 1236 ± 212 - 1229 ± 246 - 1205 ± 240	NEC ≥2	NEC 2 or 3, sepsis, TUFEF, and mortality
62	Dutta, 2015, India, 1	DB, PC	Pharma-ceutical company	27-33 wks	OMM or formula	Start before DOL 4, for 14-21 days (subgroups)	- Placebo - *B. longum*R00175($2\times10^{8-9}$ CFU), *L. helveticus* R0052 ($1\times10^{9-10}$ CFU), *L. rhamnosus*R0011($7\times10^{8-9}$CFU), and *Sa. boulardii*CNCM I-1079 ($2\times10^{8-9}$CFU);	- 35 - 114	- 30.8 ± 1.7 - 30.9 ± 1.8	- 1252 ± 309 - 1345 ± 285	Stool coloni-zation	NEC 1, NEC 2 or 3, sepsis, and mortality

							Darolac, Aristo					
63	Fernández-Carrocera, 2013, Mexico, 1	DB	Milk bank staff	<1500 g	OMM or PF	Start enteral feedings. Duration not defined	- Placebo (unsupplemented milk) - *B. infantis* (3×10^7 CFU), *L. acidophilus* (1×10^9 CFU), *L. casei* (1×10^9 CFU), *L. plantarum* (2×10^8 CFU), *L. rhamnosus* (4×10^8 CFU), and *S. thermophilus* (7×10^5 CFU); Lactipan, Italmex SA	- 75 - 75	- 31 (27-36) - 31 (26-35) $	- 1170 (540-1492) - 1090 (580-1495) $	NEC ≥2	NEC 2, NEC 3, NEC 2 or 3, sepsis, and mortality
64	Fujii, 2006, Japan, 1	-	NR	preterm infants	OMM or formula	Start several hours after birth, until discharge	- Placebo - *B. breve* M-16V (2×10^9 CFU); Morinaga	- 8 - 11	- 31.2 ± 2.0 - 31.3 ± 3.2	- 1496 ± 245 - 1378 ± 365	NR	NEC 2 or 3, and sepsis
65	Havranek, 2013, USA, 1 (sub-study of Al-Hosni,	DB, PC	Pharmacy	501-1000 g, AGA	OMM or DM	First enteral feeding, until discharge or 34 wks PMA	- Control (unsupplemented milk) - *B. longum* 35624 (5×10^8 CFU; Align) and *L.*	- 16 - 15	- 25.9 ± 1.5 - 25.9 ± 1.3	- 789 ± 129 - 856 ± 105	Superior mesenteric artery blood flow velocity	TUFEF (other outcomes already in Al-Hosni)

	2012)						*rhamnosus* GG ATCC 53103 (5×10^8 CFU; Culturelle)					
66	Hays, 2016, France, 3	DB, PC	Nurses	25-31 wks and 700-1600 g, AGA	OMM, DM (until 1500 g), or PF	Start before DOL 7, duration 4 to 6 wks	- Placebo - *B. lactis*Bb-12 (1×10^9 CFU) - *B. longum*BB536 (1×10^9 CFU) - *B. lactis*Bb-12 (1×10^9 CFU) and *B. longum*BB536 (1×10^9 CFU)	- 52 - 50 - 48 - 47	- 29.4 (27.9-30.6) - 29.0 (28.1-30.1) (all 3 probiotic groups) #	- 1170 (1055-1370) - 1170 (1000-1320) (all 3 probiotic groups) #	Weight gain	NEC 2 or 3, sepsis, TUFEF and mortality
67	Hikaru, 2010, Japan, 1	DB, PC	Dieticians not involved in clinical care	<1500 g, Survival DOL 3	OMM or PF	Start within hours after birth, until discharge	- Control (unsupplemented milk) - *B. breve* M-16V (1×10^9 CFU); Morinaga	- 100 - 108	- 28.5 ± 2.6 - 28.1 ± 2.8	- 1066 ± 272 - 1009 ± 267	NR	NEC 2 or 3*, sepsis and mortality
68	Indrio, 2017, Italy, 2	DB, PC	Pharma-ceutical company	< 37 wks, AGA, no LOS	PF	Start with enteral feeds, until DOL 30	- Placebo - *L. reuteri* DSM 17938 (1×10^8 CFU); BioGaia	- 30 - 30	- 30.1 ± 1.2 - 30.2 ± 1.2	- 1407 ± 536 - 1472 ± 455	Cytokine fecal profile	TUFEF
69	Jacobs, 2013,	DB, PC	Pharmacy	<32 wks and	OMM or formula	Start before	- Placebo - *B. infantis*	- 551	- 27.8 ± 2.0	- 1048 ± 260	Sepsis	NEC 2 or 3, sepsis, TUFEF,

	Australia, 8, New Zealand, 2			<1500 g		DOL 3, until discharge or term	Bb-02 96579 (3×10^8 CFU), *B. lactis* Bb-12 15954 (3.5×10^8 CFU), and *S. thermophilus* TH-4 15957 (3.5×10^8 CFU); ABC Dophilus	- 548	- 27.9 ± 2.0	- 1063 ± 259		and mortality
70	Kanic, 2015, Slovenia, 1	-	NR	<1500 g	OMM or PF	Start NR, until discharge	- Control - *B. infantis* PTA-5843 (4.5×10^6 CFU), *E. faecium* PTA-5844 (3×10^6 CFU), and *L. acidophilus* ssp *gasseri* PTA-5845 (4.5×10^6 CFU); Linex'	- 40 - 40	- 29.0 (26.2-30.0 - 28.0 (27.0-30.0) #	- 1024 ± 250 - 1104 ± 233	NR	NEC 2 or 3, sepsis, and mortality
71	Lin, 2005, Taiwan, 1	DB	Breast milk team	<1500 g, survival DOL 7	OMM or DM	Start feeding, until discharge	- Control (unsupplemented milk) - *B. infantis* ATCC 15697 (1×10^9 CFU/kg) and *L.*	- 187 - 180	- 28.2 ± 2.5 - 28.5 ± 2.5	- 1071 ± 243 - 1104 ± 242	NEC ≥2 or death	NEC 2, NEC 3, NEC 2 or 3, sepsis, and mortality

							acidophilus ATCC 4356 (1×10^9 CFU/kg); Infloran					
72	Lin, 2008, Taiwan, 7	DB	Breast milk team	<34 wks and <1500 g	OMM or mix of OMM and PF	Start feeding, duration of 6 wks	- Control (unsupplemented milk) - *B. bifidum* NCDO 1453 (1×10^9 CFU/kg) and *L. acidophilus* NCDO 1748 (1×10^9 CFU/kg); Infloran Berna	- 217 - 217	NR	- 1077 ± 214 - 1029 ± 246	NEC ≥2 or death	NEC 2, NEC 3, NEC 2 or 3, sepsis, and mortality
73	Manzoni, 2006, Italy, 1	DB	Human milk bank team	<1500 g, DOL 3	OMM or DM	Start DOL 3, until 6 wks of age	- Control (unsupplemented milk) - *L. rhamnosus* GG ATCC 53103 (6×10^9 CFU); Dicoflor 60	- 41 - 39	- 29.3 ± 4 - 29.6 ± 5	- 1174 ± 340 - 1212 ± 290	Enteric fungal coloni-zation	NEC 2, NEC 3, NEC 2 or 3, sepsis, TUFEF, and mortality
74	Manzoni, 2009, Italy, 11	DB, PC	Local pharmacy	<1500 g, < DOL 3	OMM or PF	Start DOL 3, until 4 to 6 wks of age	- Lactoferrin - Lactoferrin and *L. rhamnosus* GG ATCC	- 153 - 151	- 29.6 ± 2.5 - 29.8 ± 2.8	- 1142 ± 244 - 1138 ± 253	LOS	Sepsis and mortality

							53103 (6×10^9 CFU); Dicoflor 60					
75	Manzoni, 2014 Italy, 11 (extension of Manzoni 2009)	DB, PC	Local pharmacy	<1500 g, < DOL 3	OMM or PF	Start DOL 3, until 4 to 6 wks of age	- Lactoferrin - Lactoferrin and *L. rhamnosus* GG ATCC 53103 (6×10^9 CFU); Dicoflor 60	- 247 - 238	- 29.7 ± 2.5 - 29.6 ± 2.8	- 1158 ± 251 - 1129 ± 242	NEC ≥2	NEC 2 or 3, and TUFEF
76	Mihatsch, 2010, Germany, 1	DB, PC	Pharma-ceutical company	<30 wks	OMM or PF	Start when fed milk, until 6 wks of age	- Placebo - *B. lactis* Bb-12 (1.2×10^{10} CFU); Nestlé	- 89 - 91	- 26.7 ± 1.7 - 26.6 ± 1.8	- 871 ± 287 - 856 ± 251	LOS	NEC 2 or 3, sepsis, TUFEF, and mortality
77	Millar, 1993, England, 1	DB	NR	≤33 wks	OMM or PF	Start when fed milk, until 2 wks of age	- Control (unsupplemented milk) - *L. rhamnosus* GG ATCC 53103 (2×10^8 CFU); Valio Finnish Co-operative Dairies Association	- 10 - 10	- 30.0 (24-33) - 30.5 (26-33) $	- 1500 (830 – 2150) - 1445 (800-2560) $	NR	Sepsis
78	Mohan, 2006/08, Germany, 1	DB, PC	Pharma-ceutical company	<37 wks	OMM, DM, or PF	Start DOL 1, until DOL 21	- Control (unsupplemented milk) - *B. lactis* Bb-12	- 32 - 37	- 31.3 ± 2.6 - 31.1 ± 2.3	- 1398 ± 331 - 1449 ± 343	NR	NEC 2 or 3*, and sepsis*

							(4.8×10^9 CFU); Nestlé					
79	Oncel, 2014, Turkey, 1	DB, PC	Local pharmacy	≤32 wks, ≤1500g, survival DOL 7	OMM or PF	Start at first feed, until discharge	- Placebo - *L. reuteri* DSM 17938 (2×10^8 CFU); BioGaia AB	- 200 - 200	- 27.9 ± 2.5 - 28.2 ± 2.4	- 1048 ± 298 - 1071 ± 274	NEC ≥2 or death	NEC 2 or 3, sepsis, TUFEF, and mortality
80	Pärtty, 2013, Finland, 1	DB, PC	Study nurse not involved in clinical care	32-36 wks and >1500 g	OMM or PF	Start within DOL 3, duration 60 days	- Placebo - *L. rhamnosus* GG ATCC 53103 (1-2×10^9 CFU); Mead Johnson & Co, Indiana, USA	- 32 - 31	- 34.6 (32-36) $ (all infants together)	- 2393 (1550-3965 $ (all infants together)	NR	Sepsis*
81	Patole, 2014, Australia, 1	DB, PC	Clinical trial pharmacist	≤32+6 wks and <1500 g	OMM or DM	DOL 1 until 37 wks CA	- Placebo - *B. breve* M-16V (1.5 to 3.0×10^9 CFU); Morinaga Milk Industry	- 76 - 77	- 28 (26-29) - 29 (26-30) #	- 1025 (810-1260) - 1090 (755-1280) #	Stool coloni-zation	NEC 2 or 3, sepsis, TUFEF, and mortality
82	Reuman, 1986, USA, 1	DB	NR	<2000 g and preterm	OMM or PF	Start within DOL 3	- Control (unsupplemented milk) - *L. acidophilus* LA-5 (5×10^8 CFU); Chr Hansen Laboratory	- 15 - 15	- 30.5 ± 2.8 - 30.6 ± 2.7	- 1377 ± 344 - 1366 ± 302	Coloni-zation with antibiotic resistant organisms	Mortality

83	Rojas, 2012, Colombia, 9	DB,PC	Local pharmacist	≤2000 g and preterm	OMM or PF	Start within DOL 2, until discharge	- Placebo - *L. reuteri* DSM 17938 (1×10^8 CFU); BioGaia AB	- 378 - 372	- 32 (29-33) - 32 (30-33) #	- 1516 (1129-1750) - 1530 (1253-1750) #	LOS or death	NEC 2 or 3, sepsis, and mortality
84	Romeo, 2011, Italy, 1	Open label	NR	<2500 g and <37 wks	OMM or formula	Start within DOL 3, until discharge (max 6 wks)	- Control - *L. reuteri* ATCC 55730 (1×10^8 CFU) - *L. rhamnosus* GG ATCC 53103 (6×10^9 CFU)	- 83 - 83 - 83	- 33.3 ± 2.1 - 33.8 ± 1.8 - 33.3 ± 1.6	- 1946 ± 465 - 1999 ± 439 - 1941 ± 439	NR	NEC 2*, NEC 3*, NEC 2 or 3*, sepsis*, TUFEF*, and mortality*
85	Rougé, 2009, France, 2	DB, PC	Pharma-ceutical company	<1500 g and <32 wks	OMM, DM, or PF	Start first feeding until discharge	- Placebo - *L. rhamnosus* GG ATCC 53103 (4×10^8 CFU; Valio) and *B. longum* BB536 (4×10^8 CFU); Morinaga Milk Industry	- 49 - 45	- 28.1 ± 1.8 - 28.1 ± 1.9	- 1057 ± 260 - 1115 ± 251	Receiving 50% of nutrition enterally on DOL 14	NEC 2 or 3*, sepsis, TUFEF, and mortality
86	Roy, 2014, India, 1	DB, PC	NR	<2500 g and <37 wks; <DOL 14	OMM	Start within DOL 3, until discharge (max 6	- Placebo - *B. bifidum* (6×10^7 CFU), *B. lactis* (5×10^8 CFU), *B. longum*	- 56 - 56	- 32.2 ± 2 - 32.0 ± 2	- 1069 ± 365 - 1192 ± 341	LOS	NEC 1*, NEC 2*, NEC 3*, NEC 2 or 3*, any NEC, sepsis, and mortality

						wks)	(6×10^7 CFU), and *L. acidophilus* (6×10^8 CFU); Prowel by Alkem batch PWS3002C					
87	Saengtawesin, 2014, Thailand, 1	Open label, except for doctors*	NR	≤1500 g and ≤34 wks	OMM or PF	Start first feeding, until 6 wks of age or discharge	- Control (unsupplemented milk) - *L. acidophilus* NCDO1748 (1×10^9 CFU/kg) and *B. bifidum* NCDO1453 (1×10^9 CFU/kg); Infloran Berna	- 29 - 31	- 30.6 ± 1.8 - 31.0 ± 1.8	- 1208 ± 199 - 1250 ± 179	NR	NEC 2 or 3, sepsis*, and mortality
88	Samanta, 2009, India, 1	DB	NR	<1500 g and <32 wks	OMM	NR	- Control (unsupplemented milk) - *B. bifidum* (5×10^9 CFU), *B. infantis* (5×10^9 CFU), *B. longum* (5×10^9 CFU), and *L. acidophilus* (5×10^9 CFU)	- 95 - 91	- 30.1 ± 1.6 - 30.1 ± 1.6	- 1210 ± 143 - 1172 ± 143	NR	NEC 2 or 3, sepsis, TUFEF, and mortality

89	Sari, 2011, Turkey, 1	DB	Personnel in the breast-milk team	<1500 g or <33 wks	OMM or PF	Start with first feeding, until discharge	- Control (unsupplemented milk) - *L. sporogenes* (=*Ba. coagulans*) (3.5×10^8 CFU); DMG Italia SRL	- 111 - 110	- 29.7 ± 2.4 - 29.5 ± 2.4	- 1278 ± 282 - 1231 ± 262	NEC ≥2 or death	NEC 2, NEC 3, NEC 2 or 3, sepsis, TUFEF, and mortality
90	Serce, 2013, Turkey, 1	DB, PC	Personnel in the breast-milk team	≤1500 g and ≤32 wks, survival DOL 14	OMM or PF	Start with first feeding, until discharge	- Placebo - *Sa. boulardii* CNCM I-745 (1×10^9 CFU/kg); Reflor	- 104 - 104	- 28.8 ± 2.2 - 28.7 ± 2.1	- 1126 ± 232 - 1162 ± 216	NEC ≥2, LOS, or death	NEC 2 or 3, sepsis, and mortality
91	Shadkam, 2015, Iran, 1	DB, PC	NR	< 37 wks and 1000-1800 g	OMM	Start DOL 4, until 120 mL/kg/d	- Placebo (distilled water) - *L. reuteri* (4×10^7 CFU/kg); BioGaia	- 30 - 30	- 31.0 ± 1.9 - 30.9 ± 1.9	- 1419 ± 328 - 1396 ± 235	NR	NEC 2 or 3, sepsis, and mortality
92	Shashidhar, 2017, India, 1	DB	Research nurses	750-1499 g	OMM or DM	Start with first feeding, until discharge	- Control (unsupplemented milk) - *B. longum* R00175, *L. helveticus* R0052, *L. rhamnosus* R0011, and	- 52 - 52	- 31.0 ± 2.1 - 31.2 ± 2.1	- 1190 ± 208 - 1256 ± 185	TUFEF	NEC 2 or 3, sepsis*, TUFEF, and mortality

							Sa. boulardii CNCM I-1079 (1.25×10^9 CFU); Darolac, Aristo					
93	Stratiki, 2007, Greece, 1	DB, PC	Pharma-ceutical company	27-37 wks	PF	NR	- Placebo - *B. lactis* Bb-12 (2×10^7 CFU/g milk powder); Nestlé	- 36 - 41	- 30.5 (26-37) - 31.0 (27-37) $	- 1500 (700-1900) - 1500 (900-1780) $	Sugar absorption tests	NEC 2 or 3, sepsis, and TUFEF
94	Tewari, 2015, India, 1	DB, PC	NR	<34 wks	OMM or DM	Start after stopping initial abx, until 6 wks of age or discharge	- Placebo - *Ba. clausii* O/C, N/R84, T84, Sin8 (2.4×10^9 CFU); Enterogermina	- 121 - 123	- 30.4 - 30.2	- 1347 - 1377	LOS	NEC 1, NEC 2, NEC 3, NEC 2 or 3, any NEC, sepsis, and mortality
95	Totsu, 2014, Japan, 19	DB, PC, CR	NR	<1500 g	OMM or formula	Start within DOL 2, until weight 2000 g	- Placebo - *B. bifidum* OLB 6378 (2.5×10^9 CFU); Meiji	- 130 - 153	- 28.5 ± 3.3 - 28.6 ± 2.9	- 998 ± 281 - 1016 ± 289	100 mL/kg/d enteral feeding	NEC 1, NEC 2, NEC 3, NEC 2 or 3, any NEC, sepsis, and mortality
96	Underwood, 2009, USA, 1	DB, PC	Pharmacy	750-2000 g and <35 wks	OMM or formula	Start < DOL 7; duration 28 days or discharge	- Inulin and *L. rhamnosus* GG (1×10^9 CFU; Culturelle, ConAgra)	- 30 - 31	- 29.3 ± 2.6 - 29.5 ± 2.6	- 1393 ± 363 - 1394 ± 356	Weight gain	NEC 1, NEC 2, NEC 3, NEC 2 or 3, any NEC, sepsis, and TUFEF

							- Inulin, *B. bifidum* (1×10^9 CFU), *B. infantis* (1×10^9 CFU), *B. longum* (1×10^9 CFU), and *L. acidophilus* (1×10^9 CFU); ProBioPlus DDS, UAS					
97	Van Niekerk, 2015, South Africa, 1	DB, PC	Pharma-ceutical company	<1250 g and <34 wks	OMM or DM	Start with first feeding; duration 28 days or discharge	- Placebo (HIV-) - Placebo (HIV+) - *B. longum 35624* (3.5×10^8 CFU) and *L. rhamnosus* GG (3.5×10^8 CFU); Pro-B2, C Pharm. (HIV-) - *B. longum* 35624 (3.5×10^8 CFU) and *L. rhamnosus* GG (3.5×10^8 CFU); Pro-B2, C Pharm.	- 56 - 37 - 54 - 37	- 28.7 ± 3.0 (all infants together)	- 987 ± 160 (all infants together)	NR	NEC 1, NEC 2, NEC 3, NEC 2 or 3, any NEC, sepsis, TUFEF, and mortality

							(HIV+)					
98	Wang, 2007, Japan, 2	NR	NR	Premature infants	OMM or formula	Start DOL 1, until	- Control (unsupplemented milk) - *B. breve* M-16V (3.2×10^8 CFU; Morinaga Milk Industry	- 33 - 33	- 30.4 ± 4.1 - 31.1 ± 2.8	- 1276 ± 490 - 1247 ± 393	NR	NEC 2*, NEC 3*, NEC 2 or 3*, and sepsis
99	Xu, 2016, China, 1	DB	Nursing staff not involved in daily care	30-37 wks and 1500-2500 g	PF	Start NR, duration DOL 28 or discharge	- Control (unsupplemented milk) - *Sa. boulardii* CNCM I-745 (2×10^9 CFU); Bioflor	- 49 - 51	- 33.0 ± 1.0 - 33.0 ± 0.7	- 1957 ± 51 - 1947 ± 54	Weight gain	NEC 2, NEC 3, NEC 2 or 3, and sepsis
100	Zeber-Lubecka, 2016, Poland, 1	DB, PC	NR	25-33 wks	OMM (except for 1 in each group)	Start between DOL 6 and 12, duration 6 wks	- Placebo - *Sa. boulardii* CNCM I-3799 * (2×10^9 CFU); Dierol, Sequioa	- 27 - 28	- 29.8 ± 2.7 - 30.1 ± 2.3	- 1503 ± 379 - 1538 ± 340	Gut microbiota	NEC 2*, NEC 3*, NEC 2 or 3*, sepsis*, and mortality*
101	Zhang, 2017, China, 1	open	NR	< 37 wks, < 2500 g, and AGA	OMM or formula	NR	- Mosapride, *Ba. subtilis* R0179, and *E. faecium* R0026 (0.66 g); Medilac-Vita - Mosapride	- 80 - 80	- 33.6 ± 2.4 - 33.8 ± 2.1	- 1860 ± 280 - 1840 ± 300	NR	NEC (grade not specified)** and sepsis

							and *Sa. boulardii* CNCM I-745 (0.5 g); Bioflor					

DB double blind; PC placebo controlled; CR cluster randomized; NR not reported; AGA appropriate for gestational age; DOL day of life; OMM own mother's milk; DM donor milk; PF preterm formula; PMA post menstrual age; PCA post conceptual age; CFU colony forming units; CPU cells per unit; NEC necrotising enterocolitis; LOS late-onset sepsis; TUFEF time until full enteral feeding.

* Personal communication with original authors; ** No reply from original authors upon inquiry of more details; NEC 2 or 3 was assumed in analyses

§ Median; # Median (IQR); $ Median (range)

[1] In the original manuscript it is stated they used B. bifidum, which turned out to be*B. bifidum* Bb-12, nowadays better known as *B. lactis* Bb-12

Table 2: Summary of significantly effective strains or combinations in reducing mortality, NEC grade 2 or 3, late-onset sepsis, or time until full enteral feeding.

Mortality (RR):	Number of included studies	Number of included infants	Risk ratio or mean difference (in days) with their 95% credible intervals
B. bifidum NCDO 1453 and *L. acidophilus* NCDO 1748	2	494	0.16 (0.019 – 0.74)
B. bifidum, B. infantis, B. longum, and *L. acidophilus*	1	186	0.26 (0.059 – 0.98)
B. infantis, L. acidophilus, L. casei, L. plantarum, L. rhamnosus, and *S. thermophilus*	1	150	0.090 (0.0034 – 0.70)
NEC grade 2 or 3 (RR):			
B. lactis Bb-12 or B94	5	828	0.25 (0.10 – 0.56)
L. reuteri ATCC 55730 or DSM 17938	4	1459	0.43 (0.16 – 0.98)
L. rhamnosus GG	6	1507	0.24 (0.064 – 0.67)
B. bifidum, B. infantis, B. longum, and *L. acidophilus*	2	247	0.25 (0.051 – 0.89)
B. infantis ATCC 15697 and *L. acidophilus* ATCC 4356	1	367	0.16 (0.017 – 1.0)
B. infantis Bb-02, *B. lactis* Bb-12, and *S.thermophilus* TH-4	2	1244	0.29 (0.073 – 0.78)
B. longum 35624 and *L. rhamnosus* GG	2	285	0.18 (0.020 – 0.89)
Late-onset sepsis (RR):			
B.bifidum, *B.infantis*, *B. longum,* and *L.acidophilus*	2	247	0.43 (0.18 – 0.94)
B. longum R00175, *L. helveticus* R0052, *L. rhamnosus* R0011, and *Sa. boulardii* CNCM I-1079	3	241	0.34 (0.16 – 0.66)
Time until full enteral feeding (d):			

L.reuteri ATCC 55730 or DSM 17938	3	626	-3.3 (-6.4 – -0.62)
B.bifidum, *B.infantis*, *B. longum,* and *L.acidophilus*	2	247	-4.7 (-8.6 – -0.70)
B.longum BB536 and *L.rhamnosus* GG	1	94	-10 (-16 – -3.6)